Australian Journal of Crop Science
AJCS

AJCS 15(01):137-144 (2021) ISSN:1835-2707
doi: 10.21475/ajcs.21.15.01.2963

Cover crops and multifunctional microorganisms can affect development of upland rice

Fernando Couto de Araujo[1]*, Adriano Stephan Nascente[2], Marta Cristina Corsi de Filippi[2], Mariana Aguiar Silva[1], Vinícius Silva Sousa[1] and Anna Cristina Lanna[2]

[1]Agronomy School, Goiás Federal University, Goiânia, State of Goiás, 74.690-900, Brazil
[2]Brazilian Agricultural Research Corporation (EMBRAPA), Rice and Beans Research Center, P.O. Box 179, Highway 462, km 12, Santo Antônio de Goiás, State of Goiás, 75.375-000, Brazil

Corresponding author: fernandoagrocouto@hotmail.com

Abstract

Cultivation of cover crops in the off-season and the use of multifunctional microorganisms are strategic technologies to ensure sustainability in upland rice production. The objective of this work was to determine the effect of mix of cover crops cultivated in the off-season and multifunctional microorganisms on the growth promotion of upland rice plants, under no-tillage systems conducted in the Brazilian Cerrado. The experimental design was a complete randomized blocks in a 6x2 factorial scheme with four replications. The treatments consisted of a combination of six soil covering plants 1. Fallow (control); 2. millet (*Pennisetum glaucum*) and crotalaria (*Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*); 3. millet and pigeon pea (*Cajanus cajanus*); 4. millet and *Urochoa ruziziensis*; 5. millet, *U. ruziziensis* and pigeon pea; and 6. millet and buckwheat (*Fagopyrum esculentum*)), with or without the application of coinoculants 1301 (*Bacillus* sp.) + *Azospirillum* sp. The mix of cover crops millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea recorded the largest biomass production and the highest nutrient content in the straw. Rice plants cultivated after millet + pigeon pea showed largest transpiration and stomata conductance. The application of multifunctional microorganisms contributed to an increase of 29% in the photosynthetic rates of rice plants. The highest upland rice grain yield was achieved after mix of millet + crotalaria (*Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*). The application of multifunctional microorganisms increased the mass of 1000 grains, but does not affect rice grain yield. Our results showed that cover crops significantly affected rice grain yield and the multifunctional microorganisms affected grain quality.

Keywords: *Azospirillum* sp.; *Bacillus* sp.; *Cajanus cajan*; *Crotalaria* sp.; *Fagopyrum esculentum*; *Oryza sativa*; *Pennisetum glaucum; Urochoa ruziziensis*.
Abbreviations: DAS_Days after sowing; DAE_Days after the emergency; DM_Dry material; N-NH_4^+_Ammonium; N-NO_3^-_Nitrate; N_Nitrogen; P_Phosphorus; K_Potassium; Ca_Calcium; Mg_Magnesium; Cu_Copper; Fe_ iron; Mn_Manganese; Zn_Zinc; CFU_Colony Forming Unit; A_Net photosynthesis; E_Transpiratory rate; gs_Stomatal conductance; A/E_Instant water use efficiency; IRGA_Infra Red Gas Analyzer; PGPR_Plant Growt-Promoting Bacteria; IAA_indole acetic acid; e.a_acid equivalente; MAP_Monoamonic phosphate

Introduction

Rice is one of the most important food crop worldwide with a global consumption per capita of around 57 kg year^{-1} (Nascente et al., 2019). This crop is cultivated in 159 million hectares (Mha), representing 11% of agricultural cultivated area, with an annual production of 703.8 million tons (Mt) of paddy rice. From the total area, 6 million ha is the upland rice, including 9% in Asia, 46% in Latin America including the Caribbean countries and 47% in West Africa (FAO, 2020).
The United Nations estimated a world population of 9.8 billion by 2050, which will require an increase in food production of around 70%, compared to our current production (FAO, 2018). Rice grain yield should follow the rate of population growth once rice consumption will increase (Nascente et al., 2019). In this context, Brazil plays a fundamental role, as it is one of the largest food producers in the world, especially in the Cerrado region (Martins et al., 2015), which currently accounts for more than 50% of Brazilian agricultural production (Hosono and Caruso, 2016). In order to meet the growing demand for food, increases in the production of grains, beef, vegetables and fruits must be in a sustainable way (Martins et al., 2015).
In this sense, there are the multifunctional microorganisms or plant growth promoting microorganisms that have direct and indirect mechanisms to promote better plant development (Ahemad and Kibret, 2014; Nascente et al., 2017b). Among the multifunctional microorganisms, there are the rhizobacteria that promote plant growth (PGPR). They interact with the plants and promote their growth due to the production of phytohormones and exopoly-saccharides as well as the availability of nutrients such as phosphorus and iron in the soil solution (Isawa et al., 2010).
Nascente et al. (2017b) concluded that the use of rhizobacteria BRM 32113 (*Burkholderia* sp.), BRM 32111 (*Pseudomonas* sp.), BRM 32114 (*Serratia* sp.), BRM 32112

(*Pseudomonas* sp.), BRM 32109 (*Bacillus* sp.) and BRM 32110 (*Bacillus* sp.), and *Trichoderma asperellum* (UFRA-06, UFRA-09, UFRA-12 and UFRA- 52) can benefit the physiology of rice plants, and consequently, the agronomic characteristics of this crop, such as higher content of nutrients in the aerial part, accumulation of phytomass and increased productivity. Guimarães et al. (2013) found significant responses in the production of fresh matter and root growth in rice plants inoculated with *Azospirillum* spp. In addition, Pedraza et al. (2009) found higher total nitrogen content in the grains and productivity of upland rice inoculated with *Azospirillum brasilense*.

In this sense, the use of co-inoculation (application of two or more microorganisms in the same host) can be a strategy to expand/enhance the beneficial effects of these microorganisms in agricultural systems, compared to their isolated application, contributing to the sustainable production of rice upland (Silva et al., 2019). For example, Sousa et al. (2017) found beneficial effects of using a mix of multifunctional microorganisms, *Pseudomonas fluorensces* and *Burkholderia pyrrocinia*, on the physiological performance of rice plants.

Additionally, it appears that cover plants, isolated or in mix, predecessors to the main crop, are relevant components in agricultural systems (Nascente et al., 2013a). The importance falls on several factors and one of the most essential is the improvement of the biological quality of the soil by promoting change in the microbial communities that live there (Subbarao et al., 2006; Di et al., 2016; Nascente et al., 2016). Nascente et al. (2013a, 2016) showed that the productivity of upland rice was higher when planted after millet and Nascente and Stone (2018) found higher productivity of upland rice cultivated after mixes of cover plants millet + *Crotalaria ochroleuca*, millet + *Cajanus cajan*, or millet + *Cajanus cajan* + *Urochola ruziziensis*. In other research, upland rice growing after *Crotalaria spectabilis*, *C. juncea*, *Cajanus cajan*, *Vigna unguiculata*, *Mucuna aterrima* and *Canavalia ensiformis*, had grain yield about 7% higher than when sown after fallow (Reis et al., 2017).

Thus, the use of cover crops and multifunctional microorganisms in the cultivation of upland rice can be strategic to ensure sustainability of rice production in the Brazilian Cerrado region. However, there are few scientific studies available with the simultaneously use of cover crops and multifunctional microorganisms in upland rice crop. Thus, the objective of this work was to determine the effect of cover crop mix, grown in the off-season, on the biological quality of the soil, straw production and nutrient content in this straw, and the application of multifunctional microorganisms on the upland rice development in the Brazilian Cerrado region.

Results and discussion

Biomass and nutrients concentration of cover crops straw

Significant differences were observed in the production of dry matter and in the levels of nutrients in cover crops (Table 1). The mixture of millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea provided the highest amounts of straw (10.15 and 10.93 Mg ha^{-1}, respectively), which are increase of 132% and 150% of straw in relation to fallow, respectively. The fallow treatment showed the lowest production of straw biomass, similar to the mixtures of millet + buckwheat and millet + crotalaria.

The areas cultivated with millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea were those that showed the greatest availability of nutrients for the subsequent crop, except for the chemical nutrient iron (Table 1). The highest biomass production capacity observed in the mix millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea, culminates in a higher rates of nutrient cycling, with emphasis on the highest potassium availability (132 kg ha^{-1} and 159 kg ha^{-1}, respectively).

Microorganism population in the soil and gas exchange of rice plants

The areas cultivated with millet + crotalaria showed a greater abundance of colony forming units (CFUs) during the cultivation of upland rice (Table 2).

The net photosynthesis (A) of upland rice was significantly increased (29%) when the plants were treated with multifunctional microorganisms, compared to untreated plants (Table 3). Regarding cover crops, there were no differences between the photosynthetic rates of upland rice plants cultivated after fallow; millet + crotalaria; millet + pigeon pea; millet + *Urochoa ruziziensis*; millet + *U. ruziziensis* + pigeon pea; and millet + buckwheat. The other gas exchange parameters did not show significant differences with the application of multifunctional microorganisms in upland rice plants. On the other hand, the use of cover crops, prior to the main crop, influenced the parameters of gas exchange, transpiration (E) and stomatal conductance (gs). Rice grown in fallow area had the lowest transpiratory rate and stomatal conductance; while, the rice grown in the area with millet + pigeon pea showed the highest rates.

Upland rice yield components and grain yield

The grain yield and yield components of upland rice (grains per panicle and panicle per meter), did not differ between plants treated and untreated with multifunctional microorganisms (Table 4). The mass of 1000 grains was significantly higher in plants treated with multifunctional microorganisms.

Regarding cover crops, it was verified that the mix of millet + crotalaria (*C. spectabilis*, *C. juncea* and *C. ochroleuca*) provided the highest grain yield of upland rice, followed by the treatments millet + buckwheat, millet + *U. Riziziensis* + pigeon pea, and millet + pigeon pea (Table 4). On the other hand, millet + cotralaria differed from the fallow and millet + *Urochloa ruziziensis* treatments.

Fallow treatment provided low productivity in upland rice plants (Table 4). We observed the lowest production of straw biomass and low capacity to supply nutrients for the following crop in the fallow areas. The millet + *Urochloa ruziziensis* treatment provided the lowest grain yield for upland rice.

Discussion

The higher biomass production presented by the treatments millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea was due to the high capacity for regrowth of *U. ruziziensis* after the initial rains in spring, which occurred in October (Figure 1). This associated with the presence of vegetative buds in the clumps and in the stems of this species, enabling a large accumulation of biomass (Pacheco et al., 2011).

These cover crops are well known for their outstanding biomass production under different conditions, which is important for the sustainability of the practice of no-tillage, for soil protection and for the release of nutrients for the subsequent crop, contributing to the improvement of quality of Cerrado soil that is naturally low in fertility (Pacheco et al. 2011, Moro et al. 2013, Nascente et al. 2013a, 2013b).

According to Nascente et al. (2016), millet is a cover crop with fast decomposition and release of nutrients, providing benefits for the production of the subsequent crop. *U. ruziziensis* produces deep and abundant roots, absorbing nutrients at different levels of depth in the soil profile, making them available to the surface during the straw decomposition (Pacheco et al., 2011). Pigeon pea, another legume specie, adds the benefit of biological nitrogen fixation. These results show that the use of cover crops such as millet, *U. ruziziensis* and pigeon peais a sustainable alternative for improving the physical, chemical and biological quality of the soil in the off-season. Studies also showed improvements in soil physical parameters and increased upland rice grain yield after combinations of grasses and legumes as cover crops (Nascente and Stone, 2018) and increased rice grains yield after millet, as cover crops, isolated or intercropped with *U. ruziziensis* or *C. spectabilis* (Nascente et al., 2016).

The greater abundance of colony forming units (CFUs) found in upland rice areas cultivated after millet + crotalaria may have been due to two factors alone or in combination. The exudates of the two cover crops, one grassy and the other leguminous, with different architectural roots, can promote the rhizosphere housing depending on the exudates (Vives-Peres et al., 2020). The presence of multifunctional microorganisms may have favored the abundance of CFUs, since the production of enzymes and hormones can increase the volume of root hair, and consequently, the absorption and population of bacteria. According to Filippi et al. (2011), multifunctional microorganisms can control harmful microorganisms by producing antibiotics and enzymes that degrade the cell wall, favoring the root development of plants.

It is known that photosynthesis (A) is the primary process for the production of biomass, and ultimately, for agricultural production (Taiz et al., 2014). Additionally, it is known that multifunctional microorganisms positively affect the photosynthetic capacity of upland rice plants (Souza et al., 2015; Nascente et al., 2016). Our results show that upland rice plants treated with multifunctional microorganisms showed significantly higher net photosynthesis compared to untreated plants. Diazotrophic endophytic bacteria of the genus *Azospirillum* can be associated with rice, which can assist in plant growth through nitrogen fixation (Cassán and Diaz-Zorita, 2016), auxin production (Tien et al., 1979) and other phytoregulators such as abscisic acid, cytokinins, gibberellins, ethylene and polyamines (Cassán and Diaz-Zorita, 2016), production of siderophores and phosphate solubilizers, promoting greater availability of iron and phosphorus in the soil solution, respectively (Puente et al., 2004), biocontrol of phytopathogens (Bashan and Bashan, 2010; Filippi et al. 2011), either by inducing resistance or by direct antagonism, in addition to protecting plants against soil stress, such as salinity or toxic compounds (Creus et al., 1997). Therefore, these multifunctional microorganisms may be responsible for increases in the development and productivity of upland rice plants, promoting more efficient and sustainable production. We observed that the use of cover crops significantly influenced the gas exchange parameters in upland rice plants. Corroborating this information, Whitehead and Singh (2005) concluded that tomato plants have higher photosynthetic rate and transpiration, when cultivated after *Vicia villosa* and *Trifolium incarnatum* L. in relation to fallow.

The upland rice plants treated with multifunctional microorganisms showed a mass of 1000 grains, significantly higher than untreated plants. The increase in the mass of rice grains is directly related to gains in quality and productivity, presenting itself as an important gain in the use of beneficial microorganisms in the rice crop (Bordin et al., 2014). The increase in the rate of liquid photosynthesis may have been responsible for the positive increments in the mass of 1000 grains (Alvarez et al. 2012), when beneficial microorganisms were used in the rice crop.

The treatment of millet + crotalaria may have provided greater grain productivity to rice plants due to the greater abundance of colony forming units in the soil, helping the development of rice plants, and the rapid decomposition of these cover plants, making them available quickly nutrients in the soil by decomposing the straw. Cazetta et al. (2008) evaluated cover crops in upland rice crop and found the best productivity results on pigeon pea (*Cajanus cajan*) and crotalaria (*Crotalaria juncea*) straw. Bordin et al. (2003) also reported that leguminous cover crops (*Canavalia brasiliensis* and *Crotalaria juncea*) and millet, were the treatments that provided the highest values for upland rice grain yield, compared to other grasses such as cover crops, dual purpose sorghum cv. AG-2501C (*Sorghum bicolor*) and guinea sorghum (*Sorghum bicolor* type guinea). Crotalarias are leguminous species that are likely to increase N levels in the soil due to their ability to fix nitrogen and rapid decomposition and mineralization of nutrients (Fageria, 2014).

The area without cover crops (fallow) has shown low productivity in upland rice plants. Therefore, is not recommended when sustainable development is target. In addition, they favour an increase in the weed seed bank and do not adequately protect the soil (Nascente et al., 2013a). Silva et al. (2016) also observed that the use of leguminous cover crops provided higher grain yield to rice plants than fallow. Likewise, Reis et al. (2017) reported that leguminous cover crops provided higher grain yield for irrigated rice, when compared to fallow treatment. The millet + *Urochloa ruziziensis* are two grasses and probably reduced the availability of nitrogen for rice, causing negative effects on crop productivity. Nascente et al. (2013a) also observed a reduction in rice productivity in treatments with forage *Urochola ruziziensis*, *Urochloa brizantha* and *Panicum maximum*.

Our results allow inferring that the use of cover crops mix before rice sowing was highly recommended, since it promotes several benefits for the subsequent crop, such as greater nitrogen supply through biological fixation, as observed in the millet + black bean + pigeon pea, millet + *U. ruziziensis* + pigeon pea and millet + crotalaria, greater nutrient cycling as shown in the treatment millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea. In addition, they produce high amounts of straw biomass, promoting soil and water protection in the cultivated area.

Table 1. Mass of dry matter and nutrient contents of mulch from cover crop mix before rice sowing.

Factors	MS	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Cover crops	Mg ha^{-1}	------------- kg ha^{-1} ------------					--------- g ha^{-1} ----------			
Fallow	4.38^{c}	53^{bc}	6.4^{bc}	54^{b}	11.3^{d}	8.0^{b}	16.2^{b}	1529^{b}	228^{b}	78^{b}
Millet + Crotalaria	5.90^{bc}	69^{abc}	6.0^{bc}	47^{bc}	23.3^{bc}	10.3^{b}	20.2^{ab}	4271^{ab}	162^{b}	59^{b}
Millet + Pigeon pea	6.58^{b}	90^{a}	10.6^{ab}	56^{b}	27.0^{abc}	10.3^{b}	29.1^{a}	4544^{ab}	170^{b}	77^{b}
Millet + *U. ruziziensis*	10.15^{a}	71^{abc}	10.0^{ab}	132^{a}	31.3^{ab}	17.6^{a}	21.8^{ab}	4235^{ab}	358^{a}	141^{a}
Millet + *U. ruziziensis* + Pigeon pea	10.93^{a}	80^{ab}	11.8^{a}	159^{a}	35.6^{a}	19.1^{a}	22.4^{ab}	3344^{b}	423^{a}	161^{a}
Millet + Buckwheat	5.25^{bc}	38^{c}	2.2^{c}	9^{c}	20.5^{cd}	8.5^{b}	17.9^{b}	11543^{a}	223^{b}	52^{b}
CV (%)	18.79	34.64	46.11	33.81	28.53	24.64	31.32	106.68	27.50	25.90
ANOVA (Probability of the F test)	0.001	0.067	0.017	0.001	0.004	0.001	0.164	0.192	0.001	0.001

The means followed by the same letter in the column do not differ by the t test (LSD) at p ≤ 0.05. MS: dry matter. CV: Coefficient of variation. Crotalaria, mixture of species *C. ochroleuca*, *C. spectabilis* and *C. juncea*.

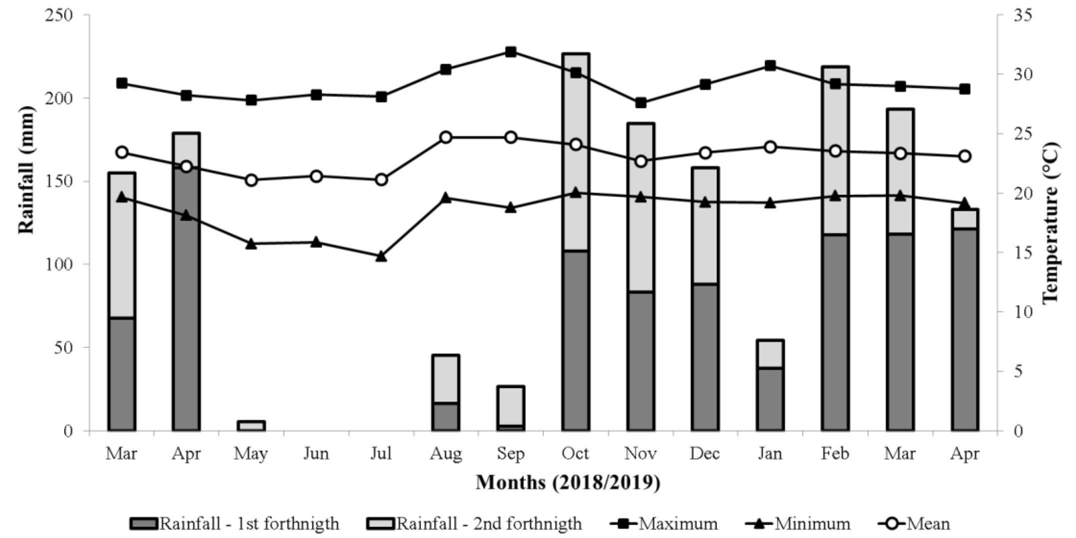

Fig 1. Air temperatures and precipitation in the first and second half of each month in Santo Antônio de Goiás, Goiás State, during the conduction of the experiment (March 2018 to April 2019).

Table 2. Effect of the cultivation of cover crops on the abundance of microorganisms in the soil.

Factors	Microorganisms
Cover crops	CFU (x10^{6})
Fallow	1.15^{abc}
Millet + Crotalaria	1.43^{a}
Millet + Pigeon pea	1.11^{bc}
Millet + *U. ruziziensis*	1.33^{ab}
Millet + *U. ruziziensis* + Pigeon pea	1.03^{c}
Millet + Buckwheat	1.06^{bc}
CV (%)	28.97
ANOVA (*p*-value)	0.0379

The means followed by the same letter in the column do not differ by the t test (LSD) at p ≤ 0.05. CFU: Colony forming units. CV: Coefficient of variation. Crotalaria, mixture of species *C. ochroleuca*, *C. spectabilis* and *C. juncea*.

Table 3. Mix of cover plants and multifunctional microorganisms affecting photosynthesis (A), transpiration (E), stomatal conductance (gs) and instant water use efficiency (A/E) of rice plants.

Factors	*A*	*E*	*gs*	*A/E*
Cover crops	µmol CO_2 m^{-1} s^{-1}	mmol H_2O m^{-1} s^{-1}	mmol H_2O m^{-1} s^{-1}	
Fallow	18.96^{a}	2.02^{b}	0.14^{b}	1.08^{a}
Millet + Crotalaria	21.25^{a}	2.85^{ab}	0.17^{ab}	1.51^{a}
Millet + Pigeon pea	22.69^{a}	3.22^{a}	0.20^{a}	1.71^{a}
Millet + *U. ruziziensis*	18.49^{a}	2.97^{a}	0.17^{ab}	1.57^{a}
Millet + *U. ruziziensis* + Pigeon pea	19.98^{a}	2.82^{ab}	0.17^{ab}	1.50^{a}
Millet + Buckwheat	25.68^{a}	3.14^{a}	0.19^{ab}	1.66^{a}
Use of microorganisms				
With	23.85^{a}	2.93^{a}	0.18^{a}	9.96^{a}
Without	18.51^{b}	2.74^{a}	0.16^{a}	9.83^{a}
Factors	ANOVA (Probability of the F test)			
Cover crops (CC)	0.5405	0.0589	0.2528	0.5242
Use of microorganisms (MICRO)	0.0343	0.2815	0.2388	0.9438
CC * MICRO	0.9166	0.6134	0.7485	0.2344
CV (%)	39.53	22.36	29.01	65.48

The means followed by the same letter in the column do not differ by the t test (LSD) at p ≤ 0.05. CV: Coefficient of variation. Crotalaria, mixture of species *C. ochroleuca*, *C. spectabilis* and *C. juncea*.

Table 4. Mix of cover crops and multifunctional microorganisms affecting productivity and production components of upland rice.

Factors	Grains per panicle	Mass of 1000 grains	Panicles per meter	Grain yield kg ha^{-1}
Cover crops				
Fallow	138^{ab}	23.7^{a}	107^{c}	4105^{bc}
Millet + Crotalaria	148^{ab}	23.9^{a}	112^{bc}	4648^{a}
Millet + Pigeon pea	152^{a}	23.8^{a}	130^{a}	4184^{abc}
Millet + *U. ruziziensis*	135^{b}	23.7^{a}	126^{ab}	3941^{c}
Millet + *U. ruziziensis* + Pigeon pea	139^{ab}	23.4^{a}	112^{bc}	4231^{abc}
Millet + Buckwheat	150^{a}	23.5^{a}	114^{bc}	4439^{ab}
Use of microorganisms				
With	146^{a}	24.1^{a}	120^{a}	4370^{a}
Without	142^{a}	23.3^{b}	114^{a}	4116^{a}
Factors	ANOVA (Probability of the F test)			
Cover crops (CC)	0.0946	0.8703	0.0233	0.0887
Use of microorganisms (MICRO)	0.3897	0.0027	0.1859	0.1226
CC * MICRO	0.7676	0.4574	0.3760	0.2337
CV (%)	10.02	3.66	12.25	11.48

The means followed by the same letter in the column do not differ by the t test (LSD) at p ≤ 0.05. CV: Coefficient of variation. Crotalaria, mixture of species *C. ochroleuca*, *C. spectabilis* and *C. juncea*.

In addition, the use of multifunctional microorganisms in the rice crop is of fundamental importance, since it promotes benefits in the physiological and agronomic aspects of the plant. This provides a higher photosynthetic rate and heavier grains that reflect in the quality of production and potential increase in income to farmers. The use of mix of cover crops, as well as multifunctional microorganisms, contribute to sustainable agricultural production.

Materials and methods

Site description

The experiment was carried out in no-tillage area under in rainfed conditions at the Capivara Farm of Embrapa Arroz e Feijão, in the municipality of Santo Antônio de Goiás, Goiás, Brazil, located at 16°29'47 ” south latitude , 49°17'20 ” west longitude and 805 m altitude, in the growing season 2018/2019. The soil in the area was classified as Acric Red Oxisol (Santos et al., 2018), with the following chemical and physical attributes in the implementation of the experiment: pH (H_2O) = 6.0; Ca mmol$_c$ dm^{-3} = 25.2; Mg mmol$_c$ dm^{-3} = 10.5; P (Mehlich) mg dm^{-3} = 9.0; K mg dm^{-3} = 137; Organic Matter g dm^{-3} = 33.85; sand g kg^{-1} = 342; silt g kg^{-1} = 164, and clay g kg^{-1} = 494, with a clayey texture. The physical and chemical analyzes of the soil were made according to the methodology proposed by Donagema et al. (2011).

The region’s climate is Tropical Savanna according to the Köppen classification (Alvares et al., 2013). Thus, there are two well-defined seasons, normally dry from May to September (autumn /winter) and rainy from October to April (spring/summer), the average annual rainfall is between 1500 to 1700 mm. The average annual temperature is 22.7°C, varying annually from 14.2°C to 34.8°C. Additionally, data on maximum, average and minimum temperatures and precipitation during the conduct of the experiment were monitored (Figure 1).

Experimental design and treatments

The experimental design was a complete randomized block in a 6x2 factorial scheme with four replications. The treatments consisted of the combination of six covering plants, 1. Fallow (control); 2. millet (*Pennisetum glaucum*) and crotalaria (*Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*); 3. millet and pigeon pea (*Cajanus cajan*); 4. millet and *Urochoa ruziziensis*; 5. millet, *U. ruziziensis* and pigeon pea; and 6. millet and buckwheat (*Fagopyrum esculentum*), with or without the application of multifunctional microorganisms 1301 (*Bacillus* sp.) + *Azospirillum*. The main plant species that developed in the fallow area were *Cenchrus echinatus*, *Euphorbia heterophylla* and *Digitaria insularis*. The plots had a dimension of 5.25 m x 10 m in length. The usable area of each plot was defined by eliminating one row of rice on each side of the plot and disregarding 0.50 m from each end. The microorganisms, in the respective treatments, were applied at three times in the crop: 1. seed microbiolization + 2. microorganism suspension sprayed on the soil 7 days after sowing (DAS) + 3. application of microorganism suspension by spraying on the part aerial view of the plant at 21 DAS. In treatments without application of microorganisms we applied water.

The bacterial isolates used are stored in the Microorganism Culture Collection at the Embrapa Rice and Bean Research Center. The biochemical characteristics and taxonomic classification of rhizobacteria are available in Nascente et al. (2017a).

Cover crops management

The area was desiccated before sowing of cover crops with spraying of glyphosate 4 L ha^{-1} (Roundup Original®, 1440 g of acid equivalent, a.e., ha^{-1}). Cover crops were sown on March 12th, 2018 in the no-tillage system, after soybean cultivation in the first harvest, using 100 kg ha^{-1} of simple superphosphate, also fertilizing the fallow area. We used mechanized seeding at a spacing of 0.45 m with a depth of 2 cm and use 10 kg ha^{-1} of millet + 20 kg ha^{-1} of seeds of another vegetable species (*U. Ruziziensis*, pigeon pea, buckwheat or crotalaria) with pure live seeds of 80% mixed in the seed distribution box of the seeder machine and sown. The cover crops were conducted following the agronomic recommendations until desiccation 20 days before sowing the rice, with an application of glyphosate (1.800 g ha^{-1} a.e.).

Multifunctional microorganisms cultivation and applications

To prepare the suspension of the bacterial microorganism, the isolates were grown in liquid medium 523 (Kado and Heskett, 1970), for 24 hours at 28°C, in a shaking incubator. The concentration of the suspension was adjusted in a spectrophotometer to an absorbance of 0.7 µm with a

wavelength of 540 nm, corresponding to $1x10^8$ colony forming units (CFU) per mL.
The microbiolization of rice seeds was carried out by immersing the seeds in each suspension for a period of 24 hours under constant agitation at 25°C and in the control treatment the seeds were immersed in water, under the same conditions. 100 mL of the suspensions of the microorganisms and the control treatment were applied directly to the soil at seven DAS and at 21 DAS the plant was sprayed, following the methodology proposed by Filippi et al. (2011).

Rice crop management

The rice cultivar A501 CL was sown in January 2019 at a row spacing of 0.35 m and density of 80 seeds per meter, using 200 kg ha^{-1} of the fertilizer monoammonium phosphate (MAP). The emergence of the plants occurred six days after sowing. At 28 (full tillering stage) and 48 (floral differentiation stage) days after emergence (DAE), nitrogen fertilizations (100 kg ha^{-1} of urea) were performed. Cultural control was carried out in order to keep the crop free from insect pests, diseases and weeds, according to agronomic recommendations.

Evaluated parameters

The straw biomass production and the chemical composition of the cover crops shoots were determined on the day of herbicide application for rice sowing. For this, a metal square with an area of 1.0 m^2 was used, randomly thrown in each plot, collected all the plants shoots and were dried in a forced air circulation oven, at 65°C for 72 hours, until constant mass, to determine the straw biomass. Subsequently, samples were taken from the straw biomass to determine the nutrient content (N, P, K, Ca, Mg, Fe, Zn, Cu and Mn) according to the recommendations established by Malavolta et al. (1997).
The microorganism population in the soil was determined in samples collected in the depth of 0-10 cm before sowing the rice and at 85 DAS. The soil samples were weighed (10g) and diluted in 90 mL of saline solution (recipe) in test tubes. After 40 minutes of stirring, each dilution of each treatment was plated in Petri dishes, containing BDA culture medium, by the plate bottom method. The plates were incubated at 25°C temperature for 48 hours and consequently in the absence of light for 7 days. The grown colonies were counted to determine colony forming units.
Gas exchange was evaluated at the time of flowering of rice plants at 85 DAS. The photosynthetic rate, A (µmol CO_2 m^{-2} s^{-1}) was evaluated; perspiration rate, E (mmol H_2O m^{-2} s^{-1}); stomatal conductance, gs (mol H_2O m^{-2} s^{-1}); and the instant efficiency in the use of A/E water (Doni et al. 2014), determined by a portable gas meter in the IRGA infrared region (LCpro, ADC BioScientific), from 8:00 am to 10:00 am. The readings were performed in the central portion of the filial limbus of the first expanded leaf (top to bottom). The equipment was configured to use concentrations of 370 to 400 mol mol^{-1} CO_2 from the air, which is the reference condition used in the IRGA photosynthesis chamber. The density of the photosynthetic photon flux was 1200 µmol m^{-2} s^{-1}. The minimum equilibrium time defined for reading was 2 min.
At 120 DAE, after the physiological maturation of the rice, the harvest was done mechanically in the useful area of each plot. The plots were evaluated for the number of panicles m^{-1}, which was determined by counting the number of panicles within 1.0 m of one of the lines in the useful area of each plot. The number of grains per panicle was determined by counting the number of grains in 10 panicles randomly sampled in the useful area and divided by 10. The mass of 1000 grains, randomly evaluated by collecting and weighing 1000 grains from each plot, corrected to 13% of the water content. The grain yield was determined by weighing the grains harvested in each plot, corrected for 13% of the water content and converted to kg ha^{-1}.

Statistical analysis

The data obtained were analyzed using the SAS statistical software (SAS, 1999). Analysis of variance (ANOVA) was performed, and when significance was detected, the LSD means comparison test was applied at the 0.05 probability level.

Conclusions

The mix of cover crops millet + *U. ruziziensis* and millet + *U. ruziziensis* + pigeon pea showed the highest straw biomass and the availability of the highest amounts of nutrients for the subsequent crop. Upland rice plants cultivated after millet + pigeon pea showed the highest transpiratory rate and stomatal conductance, while upland rice plants treated with multifunctional microorganisms significantly increased the photosynthetic rate.
The mix of millet + crotalaria provided the highest grain yield of upland rice and the application of multifunctional microorganisms contributed to the increase in the mass of 1000 grains. The cultivation of cover crops mix, as well as the use of multifunctional microorganisms can promote gains in upland rice production and contribute to sustainable production of this species.

Acknowledgments

The authors would like to thank Brazilian Agricultural Research Corporation (Embrapa) for the infrastructure for conducting the research, Goias Research Support Foundation (FAPEG) for the financial assistance in conducting the research and Brazilian Council for Scientific and Technological Development (CNPq) for the research productivity grant to the second and third authors.

References

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J King Saud Uni. 26:1-20.

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JLM, Sparovek G (2013) Köppen's climate classification map for Brazil. Meteorol Z. 22:711-728.

Alvarez RCF, Crusciol CAC, Nascente AS, Rodrigues JD, Habermann G (2012) Gas exchange rates, plant height, yield components, and productivity of upland rice as affected by plant regulators. Pesq Agrop Bras. 47:1455-1461.

Bashan Y, Bashan L (2010) How the plant growth-promoting bacterium *Azospirillum* promotes plant growth. A critical assessment. Adv Agron. 108:77-136.

Bordin L, Farinelli R, Penariol FG, Fornasieri Filho, D (2003) Double crop - commom bean with upland rice, submitted to rates of nitrogen fertilization after green cover under no-tillage system. Bragantia. 62:417-428.

Bordin LC, Casa RT, Marcuzzo LL, Sangoi L, Zancan RL (2014) Intensidade de doenças foliares, produtividade, massa de mil grãos e grãos manchados em resposta ao número de aplicações de fungicida no cultivar de arroz irrigado SCS 116 Satoru. Summa Phytopathol. 40:329-333.

Cassán F, Diaz-Zorita M (2016) *Azospirillum* sp. in current agriculture: From the laboratory to the field. Soil Bio Biochem. 103:117-130.

Cazetta DA, Arf O, Buzetti S, Sá ME, Rodrigues RAF (2008) Performance of upland rice to the nitrogen rates after different cover crops in no-till system. Bragantia. 67:471-479.

Creus C, Sueldo R, Barassi C (1997) Shoot growth and water status in *Azospirillum* inoculated wheat seedlings grown under osmotic and salt stresses. Plant Physiol Biochem. 35:939-944.

Di HJ, Cameron KC, Podolyan A, Edwards GR, Klein CAN, Dynes RA, Woods R (2016) The potential of using alternative pastures, forage crops and gibberellic acid to mitigate nitrous oxide emissions. J Soils Sediments. 16:2252-2262.

Donagema GK, Campos DVB, Calderano SB, Teixeira WG, Viana JHM (Org.) (2011) Manual de métodos de análise de solos. Rio de Janeiro: Embrapa Solos. (Embrapa Solos. Documentos, 132).

Doni F, Isahak A, Zain CRCM, Yusoff WMW (2014) Physiological and growth response of rice plants (*Oryza sativa* L.) to *Trichoderma* spp. inoculants. AMB Express. 4:45.

Fageria NK (2014) Nitrogen management in crop production. CRC Press, Boca Raton.

FAO, Food and Agriculture Organization of the United Nations (2017). Representante da FAO Brasil apresenta cenário da demanda por alimentos. Available in: <http://www.fao.org/brasil/noticias/detail-events/en/c/901168/>. Accessed 25th jul 2018.

FAO, Food and Agriculture Organization of the United Nations (2020). Statistical yearbook of the food and agricultural organization for the United Nations. Available in: < http://www.fao.org/3/i3107e/i3107e03.pdf>. Acessed 07th jan 2020.

Filippi MCC, Silva GB, Silva-Lobo VL, Cortes MMCB, Moraes AJG, Prabhu AS (2011) Leaf blast (*Magnaporthe oryzae*) suppression and growth promotion by rhizobacteria on aerobic rice in Brazil. Biol Control. 58:160-166.

Guimarães SL, Bonfim-Silva EM, Polizel AC, Moreira JCF, Rezende D (2013) Crescimento inicial de plantas de arroz inoculadas com *Azospirillum* spp. Rev Acad Cienc Agrar Ambient. 11:545-550.

Hosono A, Caruso L (2016) Agricultural transformation in the Brazilian Cerrado: A model of development and prosperity. Available in: <https://www.csis.org/events/agricultural-transformation-brazilian-cerrado-model-development-and-prosperity>. Accessed 08th sep 2017.

Isawa T, Yasuda M, Awasaki H, Minamisawa K, Shinozaki S, Nakashita H (2010) *Azospirillum* sp. strain B510 enhances rice growth and yield. Microbes Environ. 25:58-61.

Kado CJ, Heskett MG (1970) Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. Phytopathology. 60:969-976.

Malavolta E, Vitti GC, Oliveira AS (1997) Avaliação do estado nutricional de plantas: princípios e aplicações. Potafos, Piracicaba.

Martins RM, Jantalia CP, Polidoro JC, Batista J, Alves BJR, Boddey R, Urquiaga S (2015) Nitrous oxide and ammonia emissions from N fertilization of maize crop under no-till in a Cerrado soil. Soil Tillage Res. 151:75-81.

Moro E, Crusciol CAC, Cantarella H, Nascente AS (2013) Upland rice under no-tillage preceded by crops for soil cover and nitrogen fertilization. Rev Bras Cienc Solo. 37:1669-1677.

Nascente AS, Crusciol CAC, Cobucci T (2013a) The no-tillage system and cover crops – Alternatives to increase upland rice yield. Eur J Agron. 45:124-131.

Nascente AS, Crusciol CAC, Stone LF, Cobucci T (2013b) Upland rice yield as affected by previous summer crop rotation (soybean or upland rice) and glyphosate management on cover crops. Planta Daninha. 31:147-155.

Nascente AS, Filippi MCC, Lanna AC, Sousa TP, Souza ACA, Silva Lobo VL, Silva GB (2017b) Effects of beneficial microorganisms on lowland rice development. Environ Sci Pollut Res. 24:25233-25242.

Nascente AS, Filippi MCC, Lanna AC, Souza AC, Silva Lobo VL, Silva GB (2017a) Biomass, gas exchange, and nutrient contents in upland rice plants affected by application forms of microorganism growth promoters**.** Environ Sci Pollut Res. 24:2956-2965.

Nascente AS, Filippi MCC, Sousa TP, Chaibub AA, Souza ACA, Lanna AC (2019) Upland rice gas exchange, nutrient uptake and grain yield as affected by potassium fertilization and inoculation of the diazotrophic bacteria *Serratia* spp. Aust J Crop Sci. 13:944-953.

Nascente AS, Lacerda MC, Lanna AC, Filippi MCC, Silva DM (2016) Cover crops can affect soil attributes and yield of upland rice. Aust J Crop Sci. 10:176-184.

Nascente AS, Stone LF (2018) Properties and development of upland rice and soybean cultivated in rotation. Rice Sci. 25:340-349.

Pacheco LP, Barbosa JM, Leandro WM, Machado PLOA, Assis RL, Madari BE, Petter FA (2011) Produção e ciclagem de nutrientes por plantas de cobertura nas culturas de arroz de terras altas e de soja. Rev Bras Cienc Solo. 35:1787-1800.

Pedraza RO, Bellone CH, Bellone SC, Boa Sorte PMF, Teixeira KRS (2009) *Azospirillum* inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. Eur J Soil Biol. 45:36-43.

Puente M, Li C, Bashan Y (2004) Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactos seedlings. Plant Biol. 6:643-650.

Reis AFB, Almeida REM, Chagas Junior AF, Nascente AS (2017) Effect of cover crops on soil attributes, plant nutrition, and irrigated tropical rice yield. Rev Caatinga. 30:837-846.

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Araujo Filho JC, Oliveira JB, Cunha TJF (2018) Sistema Brasileiro de Classificação de Solos. Editora Embrapa, Brasília.

SAS Institute (1999) Procedure guide for personal computers. Version 5. Sas Institute, Cary.

Silva EC, Muraoka T, Franzini VI, Sakadevan K, Buzetti S, Arf O, Bendassolli JA, Soares FAL (2016) Use of nitrogen from fertilizer and cover crops by upland rice in an Oxisol under no-tillage in the Cerrado. Pesq Agrop Bras. 51:728-737.

Silva LV, Oliveira SBR, Azevedo LA, Rodrigues AC, Bonifacio A (2019) Coinoculation with *Bradyrhizobium* and *Trichoderma* alleviates the effects of salt stress in cowpea. Rev Caatinga. 32:336-344.

Sousa TP, Souza ACA, Filippi MCC, Lanna AC, Cortês MV, Pinheiro HA, Silva GB (2017) Bioagents and silicon promoting fast early upland rice growth. Environ Sci Pollut Res. 25: 3657-3668.

Souza ACA, Sousa TP, Cortês MVB, Rodrigues FÁ, Silva GB, Filippi MCC (2015) Enzyme-induced defense response in the suppression of rice leaf blast (*Magnaporthe Oryzae*) by silicon fertilization and bioagents. Int J Res Stud Biosciences. 3:22–32.

Subbarao GV, Ishikawa T, Ito O, Nakahara K, Wang HY, Berry WL (2006) A bioluminescence assay to detect nitrification inhibitors released from plant roots: A case study with *Brachiaria humidicola*. Plant Soil. 288:101-112.

Taiz L, Zeiger E, Moller IM, Murphy A (2014) Plant Physiology and Development. Sinauer Associates, Oxford University Press. 896p.

Tien T, Gaskins M, Hubbell D (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl Environ Microbiol. 37:1016-1024.

Vives-Peris V, Ollas C, Gómez-Cadenas A, Pérez-Clemente RM (2020) Root exudates: From plant to rhizosphere and beyond. Plant Cell Rep. 39:3-17.

Whitehead WF, Singh BP (2005) Fresh-market tomato gas exchange, biomass, and fruit yield are similar with legume cover crops or synthetic nitrogen fertilizer. Hortscience. 40:209-213.